OUTLINE OF MICROBIOLOGICAL METHODS

A guide to the proper collection and handling
of samples, the significance of various
microbiological parameters and the
methods used for analysis.

September 1978

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Ministry
of the
Environment

Ontario

The Honourable
Harry C. Parrott, D.D.S.,
Minister

K.H. Sharpe,
Deputy Minister

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OUTLINE OF MICROBIOLOGICAL METHODS

A guide to the proper collection and handling
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methods used for analysis.

Michael Young

MICROBIOLOGY SECTION

LABORATORY SERVICES BRANCH

MINISTRY OF THE ENVIRONMENT

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MICROBIOLOGY SECTION

The Microbiology Section conducts the analysis of water samples to determine if the microbial quality of these samples conforms to the Ministry's criteria for various water uses. Samples for these studies come from municipal drinking water distribution systems, lakes, rivers, ground water supplies and municipal and industrial wastewater disposal systems. In addition to routine samples, the Microbiology Section becomes involved in special studies of specific problems such as mercury methylation, acid lake reclamation and land runoff effects.

A number of parameters are available for the identification and evaluation of different problems. When water is being tested for its suitability from a health standpoint (for the possible presence of pathogenic bacteria), a standard membrane filtration method for total coliforms, fecal coliforms and fecal streptococci can be performed. It is also possible (with prior consultation) to determine the presence of pathogenic bacteria such as Pseudomonas aeruginosa and Salmonella sp. Total viable bacterial concentrations can be determined by the heterotrophic bacterial count (either spot or spread plate procedures).

A test developed for the analysis of drinking water is the presence-absence test which will detect the presence of coliforms, fecal coliforms, fecal streptococci, aeromonads, pseudomonads and clostridia.

Samples collected for investigation of taste, odour and colour problems are analyzed for iron bacteria, sulphur bacteria and other pertinent nuisance bacteria.

In cases where surveys call for the evaluation of specific problems, such as industrial waste inputs, additional microbial parameters can be used. These include sulphur oxidizers, sulphate reducers, nitrifiers and denitrifiers.

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Lakes & Surveys Lab	A. Qureshi	"
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PARAMETER	PREFERRED SAMPLING CONTAINER	PRESERVATION TECHNIQUE	MINIMUM VOLUME OF SAMPLE (ML)	ANALYTICAL TECHNIQUE
MICROBIOLOGY				
FECAL POLLUTION INDICATORS				
ROUTINE				
Coliforms - Total	Pre-sterilized Glass Bottles	Refrigeration (Thiosulphate with Chlorinated Water)	150	Membrane Filtration Incubation on Selective Agar
- Fecal	"	"	"	"
Fecal Streptococci	"	"	"	"
Presence Absence Procedure	"	"	"	"
Pseudomonas aeruginosa	"	"	"	"
NON-ROUTINE				
Escherichia coli	"	"	"	"
Salmonella	"	"	500 - 1000	Membrane Filtration or Moore Swabs - Incubation in Selective Broth
ORGANIC ENRICHMENT INDICATORS				
Fungi	"	"	"	Membrane Filtration Incubation on Selective Agar
Heterotrophic Bacteria - Surface Water	"	"	"	Spot or Spread Plate
- Treated Water	"	"	"	Incubation on Non-Selective Agar

PARAMETER	PREFERRED SAMPLING CONTAINER	PRESERVATION TECHNIQUE	MINIMUM VOLUME OF SAMPLE (ML)	ANALYTICAL TECHNIQUE
NUISANCE ORGANISMS	"	"	"	Direct Microscopy or MPN Incubation in Selective Broth
SPECIFIC (INDUSTRIAL/AGRICULTURAL) POLLUTION INDICATORS				
Klebsiella	"	"	"	Membrane Filtration Incubation on Selective Agar
Nitrogen Cycle Bacteria	"	"	"	MPN Incubation in Selective Broth
Sulphate Reducers	"	Refrigeration Only	"	"
Sulphur Oxidizers	"	"	"	"

SPECIALIZED CAPABILITIES Techniques for collection, handling and analysis of samples will be determined by project needs.

COLLECTION OF SAMPLES

Sterile sampling bottles are available from Central Stores in Toronto. For special studies, alternate bottles are obtainable directly from the Microbiology Section on consultation. It is the responsibility of the sampler to use aseptic techniques when handling these sterile bottles. Inaccurate results are obtained when contamination occurs through faulty handling of sterile containers.

Surface Samples

Clamp the bottle onto a sampling pole before removing the cap. Touch only the outer surface of the cap when opening the bottle. The inner lip and liner must not come in contact with anything except the atmosphere. If the inner surfaces of the cap or bottle lip are accidentally touched, the sample has been contaminated and should not be submitted. It is a recommended procedure to hold the cap with your fingertips until the sample has been taken. The cap must not be set down somewhere while the sample is being taken as this will result in contamination.

Surface sampling is accomplished by quickly lowering the sample bottle below the surface of the water and holding it there until air bubbles have stopped coming to the surface. When sampling a stream or river place the bottle with the mouth facing into the current. When sampling near shore, care should be taken to get a sample uncontaminated with sediment. The bottle is then removed from the water, the level adjusted to the top of the label, immediately capped, and then unclamped from the sampling pole. Samples must be collected using this prescribed technique. The use of a dipper or other sampling device will result in contamination.

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Depth Samples

Depth samples are taken using sterile sampling bulbs which can be obtained from the Microbiology Section in Toronto. Bulbs should be used as quickly as possible: if not used, they should be returned to the Microbiology Section within a maximum of two weeks, otherwise the rubber will crack and the bulb will not open. The same care that is used with sampling bottles must be used in the handling of bulbs. The glass plugs supplied have been sterilized within a cellophane envelope and must not come in contact with any contaminated surfaces when they are being removed from the cellophane envelope. If, for some reason, the sampler should run out of glass rods, he may dip the metal plug into alcohol and flame it. After flaming, the plug is immediately inserted into the bulb, taking the usual precautions when handling sterile equipment. The use of the metal plug is discouraged and it should only be used in rare instances when the sample could not possibly be obtained at a later time in the correct manner.

Tap Samples

Prior to sampling from a tap, the water should be allowed to run at a full flow for approximately two minutes. The strong flow will clean out residual contamination around the orfice of the tap thus providing for a more representative sample. The water pressure may then be reduced to permit taking the sample without excessive splashing which could result in contamination of the sample.

Fill the bottle to the top of the label being certain that the mouth of the bottle does not come in contact with the tap or any contaminated surface. The cap must also be handled aseptically as described previously.

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Perishability

While being transported to the laboratory, samples undergo changes (increases or decreases) in bacterial population, depending upon the initial bacterial density, the presence of growth-promoting or toxic substances in the water and the environmental conditions under which the samples are held. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

Drinking water, waste and river samples that require transportation by public carriers received 24 to 72 hours after collection can be analyzed. Samples received after three days are rejected. Negative coliform results for drinking water samples arriving more than 48 hours after collection should be considered incorrect and fresh samples should be collected.

Sample Containers and Preservation Techniques

Pre-sterilized 175 ml bottles with blue labels are usually adequate for the routine sampling of water from distribution systems, surface waters, well waters, etc. provided the waters have not been chlorinated. Chlorinated waters must be sampled in pre-sterilized 175 ml bottles containing sodium thiosulphate as a preservative (red label). Sodium thiosulphate will neutralize the disinfecting capabilities of the chlorine thereby preserving the existing microbial population at the time of sampling. Samples collected for special "nuisance" organism analyses should be collected in regular (blue label) sample bottles.

All samples should be collected early in the week and shipped to the appropriate laboratory without delay. During the Spring, Summer and Fall, samples must be packed in ice in order to minimize biological activity. During the winter, samples should be placed in an insulated container that will prevent freezing while still keeping them cold.

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Sample Volumes

In general, one bottle or bulb per sample provides sufficient volume for standard analysis. If, however, the bacterial levels expected are very low or extra parameters are being requested, then additional samples may be required. Consultation with the Microbiology Section is advisable in such cases. When a duplicate sample is being taken, it should be obtained at the same time as the first sample. This can be achieved for surface samples by clamping two bottles on a sampling pole and for depth samples by placing two bulb samplers on the sampling line in a 'piggyback' fashion.

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FECAL POLLUTION INDICATORS

ROUTINE

Total Coliforms (TC) Procedure

The TC group is comprised of all of the aerobic and facultative anaerobic, Gram-negative, non-sporeforming, rod-shaped bacteria which ferment lactose with gas formation within 48 hours at $35^{o}C$ or all organisms which produce a colony with a golden- green metallic sheen within 24 hours of incubation using the MF technique. All coliforms are oxidase negative. This definition includes, in addition to the intestinal forms of the Escherichia coli group, closely related bacteria of the genera Klebsiella, Citrobacter and Enterobacter. Enterobacter - Citrobacter groups are common in soil but are also recovered from feces in small numbers. Therefore, the presence of total colforms in water is not a specific indicator of sewage or fecal pollution but may also indicate soil runoff.

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

TC levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours (in some cases 3 days is allowed but this procedure is not advised). The necessary frequency of sampling is outlined in the MOE Guidelines and Criteria booklet (1976).

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Method

Analysis for TC is by membrane filtration according to the MOE Handbook of Analytical Methods for Environmental Samples (HAMES). A given volume of the sample or dilution is filtered using a 0.45μ gridded, white membrane filter (Gelman GN-6). The membrane is placed on m-Endo Agar LES (Difco) and incubated for 22 ± 2 hours at 35 ± 0.5°C. After incubation, the number of total coliforms and background colonies are counted using a stereoscopic microscope at a 10X magnification.

Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, the number of parameters requested per sample and the incubation periods for each parameter. TC results are generally available within 48 hours of submission.

Fecal Coliforms (FC) Procedure

This FC group of bacteria is basically a subgroup of the Total coliforms and is comprised of aerobic and facultative anaerobic, Gram-negative, non-sporeforming, rod-shaped bacteria which ferment lactose at 44.5^{o}C. The FC parameter is a more specific test for bacteria associated with human and animal fecal material. This test has proved useful as an indicator of relatively recent fecal pollution inputs, however, it is by no means completely selective for Escherichia coli, the coliform most directly related to fecal pollution. Bacteria related to other forms of input such as Klebsiella may be detected with this test.

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml - analysis performed on sample obtained for TC	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

FC levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours (in some cases 3 days is allowed but this procedure is not advised). The necessary frequency of sampling is outlined in the MOE Guidelines and Criteria booklet (1976).

Method

Analyses for FC is by membrane filtration (HAMES). The membrane is placed on a filter pad saturated with MacConkey Membrane Broth (Oxoid) and incubated at 44.5 ± 0.5^{o}C for 20 ± 1 hours. After incubation, the number of FC colonies are determined with a stereoscopic microscope at 10X magnification. All yellow, yellow-brown and yellow-green colonies are counted.

Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, the number of parameters requested per sample and the incubation periods for each parameter. FC results are generally available within 48 hours of submission.

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Fecal Streptococci (FS) Procedure

The FS group of bacteria is comprised of aerobic, spherical to ovoid Gram positive cells occurring singly, in pairs or in short or long chains. When used as indicators of fecal contamination, the following species and varieties are implied: Streptococcus faecalis, S. faecalis var. liquefaciens, S. faecalis var. zymogenes, S. faecium, S. faecium var. durans, S. bovis and S. equinus. FS can best be used in conjunction with the FC parameter to indicate the nature of the fecal source. If the ratio of the geometric mean densities of FC to FS at pollution sources or outfalls exceeds 4, then the source is likely human in origin. A ratio of 0.7 or less suggests a non-human source. Ratios between these values are difficult to interpret and may be mixtures. Numerous environmental factors may influence the densities of FC and FS and thus care must be used in interpretation. This ratio is only valid when the FC density approaches or exceeds 100/100 ml.

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml - analysis performed on sample obtained for TC and FC	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

FS levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours (in some cases 3 days is allowed but this procedure is not advisable). The necessary frequency of sampling is outlined in the MOE Guidelines and Criteria booklet (1976).

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Method

Analysis for FS is by membrane filtration (HAMES). The membrane is placed on m-Enterococcus Agar (Difco) and incubated at $35.0 \pm 0.5^{\circ}C$ for 48 ± 3 hours. Using a stereoscopic microscope (10X), all colonies that are red, maroon or pink are counted as fecal streptococci.

Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, the number of parameters requested and the incubation periods for each parameter. FS results are usually available within 72 hours of submission.

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Presence-Absence (P-A) Procedure

The P-A procedure has been developed as an alternative to the membrane filtration procedure and is a sensitive means of detecting pollution indicator bacteria in drinking water samples. Essentially, the P-A test is a modification of the most probable number (MPN) procedure, which uses a dilution series of broth tubes to detect and enumerate microorganisms. The P-A test employes only one analysis bottle per sample. The test allows for an inoculum of up to 100 ml and will qualitatively determine a wide variety of pollution indicator bacteria. They include total coliforms, fecal coliforms, fecal streptococci, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aeromonas* sp. and *Clostridium perfringens*.

Sampling

Minimum Volume of Sample	Required Sample container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

Bacterial levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within 6 hours, but not exceeding 24 hours if feasible. Sample analysis will not be performed on samples older than 3 days. The necessary frequency of sampling is outlined in the MOE Drinking Water Objectives (1976) booklet.

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Method

The P-A test involves the inoculation of 50 or 100 ml of water into a bottle of MacConkey broth (HAMES). The bottle is incubated at 35 $\pm$ 0.5^{o}C for up to four days. If growth occurs with the production of acid and/or gas, then other tests are used to determine the types of bacteria present.

Time of Analysis

The time required for completion of test procedures associated with the P-A test is up to one week depending on the level of pollution in the sample. A sample containing a variety of indicator bacteria will require several confirmation tests before results can be reported. Drinking water samples which show the presence of fecal bacteria within 24 hours will have this information phoned to the municipality as soon as confirmed results are available.

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Pseudomonas aeruginosa (P. aeruginosa) Procedure

P. aeruginosa is a motile, Gram-negative rod which does not ferment lactose and is oxidase positive.

Isolates of P. aeruginosa are found in human feces and their presence in water is generally the direct cause of recent fecal waste inputs from a nearby source. Their presence is thus a good indication of a potential health hazard.

P. aeruginosa is also an opportunistic pathogen and has been identified as the causative agent of a number of infections that may be transmitted through a contaminated water body to a susceptible host. Of chief concern to bathers is the association between P. aeruginosa and the disease known as otitis externa "swimmer's ear".

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

P. aeruginosa levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

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Method

An appropriate volume or dilution of the sample is filtered through a 0.45 u membrane filter and incubated on mPA Agar at 41.5 ± 0.5°C for 48 ±2 hours..

Using a stereoscopic microscope (10X), all tan to dark-brown or black colonies are counted as Pseudomonas aeruginosa.

Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, the number of parameters requested and the incubation periods for each parameter. Consultation is necessary prior to this parameter being requested. P aeruginosa results are usually available 72 hours after submission.

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NON-ROUTINE

Escherichia coli (E. coli) - Experimental Procedure

E. coli is the predominant, facultative bacterial species in the large bowel and is thus the coliform most directly related to fecal pollution. E. coli is occasionally pathogenic to man (e.g. urinary tract infections) but its primary importance in water bacteriology is as an indicator.

Methods developed historically for the detection of fecal pollution have not been sufficiently specific for E. coli. However, a recently developed medium designed specifically for the detection of E. coli has been tested in this laboratory and proven successful. It is now possible to determine concentrations of E. coli on a limited number of samples.

This technique can only be performed with prior consultation and is not available for routine work.

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water.

E. coli levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

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Method

Analysis for E. coli requires filtration of an appropriate volume or dilution of the sample and incubation on mTEC Agar. The plates are incubated at 35 ± 0.5°C for two hours followed by 22 ± 1 hours at 44.5 ± 0.5°C. Using a stereoscopic microscope (10X), all colonies that are yellow are counted as E. coli.

Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, and the incubation period. E. coli results will be available 2 days after sample submission.

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Salmonella

Salmonella is one of the most important and common waterborne pathogenic organisms. The members of this genus are widely distributed in the environment and are probably responsible for most of the recognized waterborne disease outbreaks. Salmonella can be frequently detected in sewage, stormwater and polluted rivers and lakes. Fecal contamination from animals, particularly domestic, and insufficiently treated human wastes are the major source of these organisms.

Since salmonella serotypes are all potentially pathogenic, their frequent isolation is taken as proof of a public health hazard.

The methods required are complex and slow, therefore their use is recommended only for specific studies and only with careful consultation and planning.

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
500-1000 ml (18-36 ounces)	Pre-sterilized glass bottles	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

Sampling can also be carried out with Moore Swabs (gauze pad) placed at appropriate locations in sampling areas.

Salmonellae levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

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Method

There are several procedures for the isolation and identification of salmonellae from waters and wastewaters. The method chosen will depend on the survey conducted. All the procedures include sample concentration (Moore swab or membrane filtration), enrichment in selective broths, plating on selective agar media and identification by a variety of biochemical and serological tests.

Time of Analysis

The number of samples analyzed will depend on the volume and type of sample and, more specifically, on the methods necessary for analysis. In general, a five-day period is required for *Salmonella* analyses.

Consultation with Microbiology staff prior to the use of this parameter is absolutely essential.

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ORGANIC ENRICHMENT INDICATORS

Fungi (Yeasts and Molds)

Fungi are ubiquitously distributed, achlorophyllous, heterotrophic microorganisms which are present wherever living or nonliving organic matter occurs. Both saprophytic and pathogenic fungi have been found in sewage and sludge, domestic and industrial effluent and marine and freshwater environments. The densities and distribution of species of fungi in such habitats can be used as an index of the degree of organic enrichment. In addition, the presence of certain pathogenic fungi (e.g. Candida albicans) in recreational waters is useful in assessing water quality and in evaluating public health hazards.

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

Fungal levels, yeasts in particular, will undergo change even upon refrigeration, therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

Method

No standard techniques have been developed for quantitative enumeration of fungi from an aquatic environment. In general, selective agar media, the membrane filtration technique and the spread plate technique are used. Fungal densities are expressed as colony-forming units (CFU) per 100 ml.

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Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required and the incubation periods. In general, the analysis requires a five-day period. Prior consultation is required before this parameter can be requested.

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Heterotrophic Bacteria (HB) - Surface water

The HB count is not a test for a specific microorganism. It is designed to enumerate as large a number as possible of those bacteria in water that require some organic carbon for their growth. The concentration of heterotrophic bacteria in water is affected by the levels of organic and inorganic nutrients present. Densities of HB in lakes of high trophic status are higher than HB levels in oligotrophic lakes.

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
Can generally be done from sample for analysis of TC, FC and FS or P. aeruginosa (150 ml)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

HB levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

Method

Analysis is by the spot plate or spread plate method. Two volumes of 0.1 ml of the sample and/or sample dilution are placed on pre-dried CPS agar plates. The spread plate method requires one volume of 0.1 to 1.0 ml spread over the surface of the pre-dried agar plate. Plates are incubated at 20 ± 0.5°C for 7 days. Using a stereoscope microscope (10X), a total colony count is obtained.

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Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, and the incubation period. HB results by this method are available 8 days after sample submission.

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Heterotrophic Bacteria (HB) - Treated Water

The HB parameter can also be used for the analysis of treated waters (i.e. drinking waters including treated surface waters, water in distribution systems, bottled water, untreated well water and bathing waters including treated beaches, pools, saunas and mineral springs). In these waters the HB parameter is used to measure treatment efficiencies and as an indicator of bacterial water quality. This procedure has not yet been introduced as a routine method and thus prior consultation is required before it is requested.

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
Can generally be done from sample analysis of TC, FC and FS or P. aeruginosa (150 ml)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

HB levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, peferably within six hours, but not exceeding 24 hours.

Method

Analysis is by the Spot Plate, Spread Plate, or membrane filtration if concentrations of HB are low. Standard Methods (14th Edition) recommends the use of tryptone glucose extract or plate count agar with an incubation period of 48 ± 3 hours (bottled water for 72 ± 4 hours) at 35 ± 0.5°C. A total colony count is obtained using a stereoscopic microscope (10X).

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Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required and the incubation period. HB results by this method would be available 3 to 4 days after the sample is submitted.

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NUISANCE ORGANISMS

Certain microorganisms, although not pathogenic or indicative of the presence of fecal material, may be related to a deterioration in water quality or may reduce the efficiency of the effective treatment of water or sewage. Foul tastes and odours, slimes, discolouration, bulking of activated sludge, and the clogging or corrosion of treatment or distribution systems may all result from the presence of one or more groups of microorganisms. Nuisance organisms include iron bacteria such as Gallionella, Leptothrix, Sphaerotilus and Crenothrix, the sulphate reducing bacteria of the genera Desulfovibrio and Desulfotomaculum, the sulphur oxidizer Beggiatoa, actinomycetes, algae and fungi.

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water*

* Do not use thiosulphate bottles when presence of sulphate reducers is suspected. The sample should contain material representative of the problem such as slimes or filamentous growth collected aseptically.

Nuisance organism levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 48 hours.

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Method

With the exception of the sulphate reducing bacteria and fungi, identification is generally based upon direct microscopic observation of the sample. Morphological characteristics are generally used to identify the organism(s) present. The sulphate reducers may be cultivated and their presence revealed by the detection of metabolic byproducts. Fungi may also be cultivated and later identified by microscopic observation of their morphological characteristics.

Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of parameters requested and the incubation periods (if necessary) for each parameter. Results of microscopic analyses are available two days after sample submission, however, because the method and number of analyses vary with the sample problem, some analyses make take one month to complete. Consultation with Microbiology staff is necessary prior to sample collection.

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SPECIFIC (INDUSTRIAL/AGRICULTURAL) POLLUTION INDICATORS

Klebsiella pneumoniae (K. pneumoniae) - Experimental Procedure

K. pneumoniae are frequently found in the respiratory, intestinal and urogenital tracts of man, but these organisms may be isolated from a variety of animals and materials and are well distributed in the environment. It is associated primarily with water high in organic matter. Thus, K. pneumoniae can be used to determine the extent of influence of certain industrial wastes. A further concern, which still has to be proven epidemiologically, is that K. pneumoniae which is an opportunistic pathogen, could infect humans and cause a highly fatal type of pneumonia which accounts for 3% of bacterial pneumonias.

This is a newly developed technique which can only be performed on a limited number of samples and is not available for routine work.

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

K. pneumoniae levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

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Method

Analysis for Klebsiella pneumoniae is by membrane filtration of an appropriate volume or dilution of a sample with incubation on mK Agar at 35 ± 0.5°C for 48 ± 3 hours. Using a stereoscopic microscope (10X), all non-red colonies larger than 2 mm in diameter are counted as Klebsiella.

Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, and the incubation period. K. pneumoniae results will be available 3 days after sample submission. Prior consultation is required before a request for analysis can be considered.

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Nitrogen Cycle Bacteria

Several groups of bacteria contribute extensively to the transformations occurring within the nitrogen cycle , both in aquatic and terrestrial environments. These groups include: a) the ammonifiers, comprised mainly of heterotrophic bacteria, which release ammonia from decaying proteinaceous material; b) the nitrifying bacteria, comprised of the genera Nitrosomonas and Nitrobacter, which, by an autotrophic, aerobic process oxidize ammonia to nitrite and nitrite to nitrate; c) the denitrifying bacteria, a diverse bacterial group, which through mainly anaerobic processes, remove nitrate from the environment either by reduction to nitrite or ammonia, or by transformation of nitrate to nitrogen gas; and d) the nitrogen-fixing bacteria, i.e. Azotobacter, which transform nitrogen gas into ammonia nitrogen.

The detection and enumeration of these bacteria in water and wastewaters are not necessarily used as an indication of deteriorating water quality. However, elevated levels of nitrifying bacteria may indicate excessive loading with ammonia-nitrogen and an associated increased oxygen demand. Their enumeration, complemented by activity studies, reflects the potential of an aqueous environment to mineralize proteinaceous nitrogen (ammonifiers, nitrifiers and denitrifiers) or the ability to supplement nitrogen requirements through nitrogen fixation.

Methods presently available in the MOE are only for the enumeration of nitrifying and denitrifying bacteria.

Method

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

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Nitrogen cycle bacterial levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, but not exceeding 48 hours.

Method

Nitrogen cycle bacteria are determined by MPN analyses in selective media broths. Incubation for the nitrifiers is at 28 ± 0.5°C for 28 days under aerobic conditions. Denitrifiers are incubated anaerobically at 20 ± 0.5°C for 21 days.

Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, the number of parameters requested and the incubation periods for each parameter. Nitrosomonas and Nitrobacter results are available 29 days after sample submission. Denitrifier results are available in 22 days. Prior consultation with Microbiology Section staff is necessary before requesting these parameters.

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Sulphate-Reducing Bacteria

The sulphate-reducing bacteria (e.g. Desulfovibrio, Desulfotomaculum) are a group of organisms which obtain energy from the reduction of sulphate to sulphide and grow in anaerobic environments which contain organics and a supply of sulphate. The activity of these organisms conntributes to the deterioration of water quality by their production of H_2S and their capability of maintaining anaerobic and reducing environments.

The organisms have an economic importance in their contribution to the corrosion of iron pipes and steel containers, spoilage of stored petroleum products and the blackening of wood pulp.

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Do not use bottles containing thiosulphate.

Sample containers should be as full as possible and tightly sealed to prevent oxidation. Do not leave an air space.

Sulphate reducer levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 48 hours.

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Method

Sulphate reducers are determined by a Most Probable Number (MPN) technique. Using tubes of API broth with tryptone, a 3 x 3 series is set up using three appropriate volumes (weights) and/or dilutions of the sample (e.g. 3 tubes each of 10 ml, 1 ml and .1 ml). The tubes are layered with paraffin oil, to provide anaerobic conditions and incubated for 21 days at 28 ± 0.5°C. A black precipitate is taken as a positive reaction.

Time of Analysis

The number of analyses performed per day will depend on the type of sample and the number of dilution steps required. Sulphate reducer results are available 22 days after sample submission. Prior consultation with Microbiology Section staff is required before this parameter can be requested.

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Sulphur-Oxidizing Bacteria

The sulphur-oxidizing bacteria of the bacterial genus Thiobacillus are a group of aerobic, autotrophic organisms (T. thioparus, T. thiooxidans and T. ferrooxidans) which derive energy from the oxidation of elemental sulphur, sulphide or thiosulphate. These organisms are able to multiply and maintain acidic environments at pH values as low as 1.5. They contribute to the deterioration of water quality by lowering the water pH when the water body is enriched with reduced sulphur compounds and are responsible for the formation of acidic conditions in leachates of mine-tailings containing sulphide-based ores. Their activity also contributes to the corrosion of concrete in sewers and structures immersed in water.

Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Do not use bottles containing thiosulphate

Sulphur oxidizer levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, but not exceeding 48 hours.

Method

Sulphur-oxidizing bacteria are determined by an MPN method. Media most often used by the MOE are thioparus, thiooxidans and ferrooxidans broth. Incubation is under aerobic conditions at 28 ± 0.5°C for 21 days.

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Time of Analysis

The number of analyses performed per day will depend on the type of sample and the number of dilution steps required. Sulphur oxidizer results are available 22 days after sample submission. Prior consultation with Microbiology staff is necessary before this parameters is requested.

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SPECIALIZED CAPABILITIES

A major function of the Microbiology Section is the analysis of parameters for detecting the presence and degree of specific microbial pollutants (previous sections). In addition to the above function, there is available a high degree of specialized expertise and equipment for conducting project work designed to provide answers for specific microbial problems.

Procedures available include biomass determinations (direct count, ATP and Coulter counter), metabolic activity (respirometer, continuous culture and (AMP + ADP)/ATP) and microbial transformation of environmental contaminants (batch culture, continuous culture, respirator studies). Projects can be designed to cover most areas involving microbes including industrial treatment problems, sediment microbiology, microbial modelling and solid waste problems. The Microbiology Section is also developing a capability to perform the Ames technique (mutagenicity assay) and to perform virological analyses.

Such specialized work requires a great deal of planning and is time-consuming. It can not be considered as part of the routine work and would require specific consultations and funding. However, such projects are of great value in obtaining essential information on most microbiologically related problems.